Chapter 1
Metallomics: The Science of Biometals and Biometalloids

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Abstract Metallomics, a discipline integrating sciences that address the biometals and biometalloids, provides new opportunities for discoveries. As part of a systems biology approach, it draws attention to the importance of many chemical elements in biochemistry. Traditionally, biochemistry has treated life as organic chemistry, separating it from inorganic chemistry, considered a field reserved for investigating the inanimate world. However, inorganic chemistry is part of the chemistry of life, and metallomics contributes by showing the importance of a neglected fifth branch of building blocks in biochemistry. Metallomics adds chemical elements/metals to the four building blocks of biomolecules and the fields of their studies: carbohydrates (glycome), lipids (lipidome), proteins (proteome), and nucleotides (genome). The realization that non-essential elements are present in organisms in addition to essential elements represents a certain paradigm shift in our thinking, as it stipulates inquiries into the functional implications of virtually all the natural elements. This article discusses opportunities arising from metallomics for a better understanding of human biology and health. It looks at a biological periodic system of the elements as a sum of metallomes, and focuses on the major roles of metals in about 30-40% of all proteins, the metalloproteomes. It emphasizes the importance of zinc and iron biology, and discusses why it is important to investigate non-essential metal ions, what bioinformatics approaches can contribute to understand metalloproteins, and why metallomics has a bright future in the many dimensions it covers.

Keywords biometals, biometalloids, bioinformatics

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Abbreviations

AAS	atomic absorption spectroscopy
BMP	basic metabolic panel
CMP	comprehensive metabolic panel
ICP-MS	inductively coupled plasma mass spectrometry
MRI	magnetic resonance imaging
SMA	sequential multiple analysis

1.1 Introduction

Similar to genes/proteins, genomes/proteomes and genomics/proteomics, and any other trilogy applied to biomolecules, one can define the relationship among metals, metallomes, and metallomics. The late RJP "Bob" Williams used the word *metallome* to describe the variety of pathways for distributing individual chemical elements (metals) that are responsible for the specific character of an organism (Williams 2001). However, it was the inspiration and foresight of Hiroki Haraguchi, now professor emeritus, Nagoya University, to coin the word *metallomics* in order to establish an integration of biometals sciences and to highlight the significance of metal ions for life. Metallomics offers many opportunities to advance science by overcoming the limitations of individual and scattered disciplines that traditionally address only some aspects of metals in biology (Haraguchi 2004; 2017). It is a holistic approach that aims at describing functions of entire biological systems from an understanding of the complexities arising from the interactions of its parts. It emphasizes quantitative aspects and often includes multidisciplinary investigations.

The definitions of "omes and omics" has become a fad in biology. An editorial discussed the instances where the suffixes are useful, separating "goodomes" from "badomes" (Baker 2013). The former provide (i) a new focus and do not just rename existing fields, (ii) a comprehensive collection instead of describing an area with limited scope, (iii) an easy-to-pronounce and easy-to-understand term rather than a garbled or obscure word. The editorial specifically mentions that the terms metallome and metallomics fulfil the criteria for goodomes/goodomics.

In an IUPAC (International Union of Pure and Applied Chemistry) publication, the following definitions and three notes have been given (Lobinski et al. 2010).

"**Metallome**: Entirety of metal- and metalloid species present in a biological system, defined as to their identity and/or quantity.

1 The metallome can be determined in a bulk biological sample representative of the system [or its component(s)] or at specific location(s).

2 The metallome can be characterized with different degrees of approximation, such as a set of (i) total element concentrations; (ii) metal complexes with a given class of ligands, e.g. proteins or metabolites, or (iii) all species of a given element, e.g. the copper metallome

3 In contrast to the genome of which the analysis has a specific endpoint (…) the description of a metallome…can never be complete. In particular, the numerous … metal complexes with biological ligands can be described only in terms of kinetic constants and defined thermodynamic equilibria.

Metallomics: Study of the metallome, interactions, and functional connections of metal ions and other metal species with genes, proteins, metabolites, and other biomolecules in biological systems. A metallomics study is expected to imply: (i) a focus on metals (…) or

metalloids (…) in a biological system, (ii) a link between the set of element concentrations or element speciation with the genome. This link may be statistical (…), structural (…) or functional (…); or (iii) a systematic or comprehensive approach. The identification of a single metal species, however important, without specifying its significance and contribution to a system should not be referred to as metallomics."

Two aspects are worth adding:

a) Per above definitions *metallomes* refer to biological systems. However, metallomes also can be investigated in relation to the environment of biological systems, in water (hydrosphere), soil (lithosphere), and air (atmosphere) (Haraguchi 2004), generating knowledge of how the connection between the geosphere and the biosphere comes to bear on biological systems.

b) There is a semantic issue, namely that the word *metal* refers to a chemical element. After all, the methods employed to analyse metals in biology, such as atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectrometry (ICP-MS) are methods of elemental analysis – in contrast to the more common use of mass spectrometry as a method of molecular analysis. In biology, however, the ionic state and not the elemental state of the metal is the one that is almost always functionally important. To overcome this limitation of the words metallome and metallomics, the terms *ionomes* and *ionomics* were introduced in work on systematic elemental profiling of minerals and trace elements in plants. The terms include metals, metalloids and non-metals (Lahner et al. 2003). The ionome as one of the "four basic biochemical pillars of functional genomics", namely the transcriptome, proteome, metabolome, and ionome, draws attention to the fact that the information of the elemental composition of an organism is encoded in the genome in terms of the proteins that coordinate the acquisition and distribution of the chemical elements. The functional

interactions of the ionome with the other three basic pillars are critical for understanding the biochemical basis of life (Salt 2004). These interactions can be direct, for example when a metal ion affects the structure and function of a protein, or indirect, for example when a metal ion affects the expression of proteins.

Included in the above definition of metallomics are metals and metalloids; none of the latter has been shown to be essential for humans, though. Excluded are a number of non-metals that are also essential for life. Another word in lieu of ionomics is *elementomics*. Clearly, not all chemical elements occur in an ionic form, as they can also form covalent compounds as in the case of iodine or selenium, for example. It includes all the chemical elements (Li et al. 2008). Since metal ions and non-metals are metabolites, the discipline has also been referred to as elemental metabolomics (Zhang et al. 2017).

The information gathered in metallomics is not only that of the type of ions but also includes the identification of their valence states and coordination environments, namely speciation analysis in qualitative and quantitative terms, which can be addressed by combining elemental and molecular mass spectrometry with hyphenated techniques (Szpunar 2004). Investigations, therefore, address the presence of metal ions in different oxidation states, the binding of metal ions to macromolecules, where interactions with proteins dominate (metalloproteomes), and the interaction of metal ions with low molecular weight compounds. For example, when we talk about "iron biology" we neither specify the valence state of iron nor its coordination environment. Iron in biology refers to iron ions, Fe^{2+} (ferrous), Fe^{3+} (ferric) or even higher oxidation states in intermediates of enzymatic reactions. This distinction is important because the chemistries of Fe(II) and Fe(III) are very different in terms of reactivity and the stability of complexes. In the case of chromium, Cr(III) is a cation and supposedly essential while Cr(VI) occurs as chromate, an oxyanion, which is toxic and a carcinogen. Therefore Cr(III) is expected to compete with cations such as Fe(III) whereas

Cr(IV) competes with anions such as sulphate or phosphate. In zinc biology, however, only one valence state, Zn(II), is important. Yet, the coordination chemistry of zinc in biology is quite exquisite, dynamic, and protein-bound zinc as well as non-protein bound zinc ions are functionally important (Maret and Li 2009).

Last but not least, a reflection on the meaning of the term *biometals* is useful. The term refers to the metals that are present in or important for organisms. It is appropriate because with the high sensitivity of modern ICP-MS instrumentation it became possible to measure almost all chemical elements in organisms. This finding constitutes a certain shift of paradigm because it indicates that we should not focus only on the metals that are essential for a species but also on the ones that are non-essential because metal ions are reactive and their presence has functional consequences. When extending the concept of biometals to essential and non-essential non-metals, it is appropriate to use the term *bioelements*. A speciation and metalloproteomics approach amplified the notion that non-essential elements are functionally significant and indicated the extent of uncharted territory (Cvetkovic et al. 2010), namely that the metalloproteome of the microorganism *Pyrococcus furiosus* is largely uncharacterized. Specifically, out of the 343 metalloproteins characterized, 158 were recently unknown, including new nickel and molybdenum proteins but also proteins that contain lead and uranium. This experimental approach also shows the limitations of bioinformatics approaches to predict whether or not proteins are metalloproteins as discussed later in this article. Such predictions are possible on the basis of knowledge for essential metal ions and their coordination environments but can only gauge the possible interaction of non-essential metal ions with proteins in sites that normally bind essential metal ions.

In this article, I will discuss why an integration of the different fields dealing with biometals is useful, and in fact desirable, and point out the potential of metallomics for advancing science, using human biology as an example. A more comprehensive text on how

metallomics bridges the biology and chemistry of biometals has been published (Maret 2016a).

1.2 History of Biometals Sciences

Metal ions in biology are investigated in many different fields. In nutrition, the focus was – and remarkably still is – to define the elements that are essential for life. In toxicology, the adverse effects of metal ions on biological structure and function are investigated, and in pharmacology, their therapeutic effects. The three disciplines cover different ranges of the biological dose response curve: nutrition mainly the lower concentration range up to the range where functional responses are optimal, pharmacology the range where an enhancement of functions is observed, generally supraphysiological concentrations, and toxicology the range of even higher concentrations. Whereas nutrition is primarily concerned with essential metal ions, pharmacology and toxicology address non-essential metal ions as well. However, this separation is arbitrary. In nutrition, the presence of non-essential elements affects the function of essential metal ions and in toxicology the presence of essential elements affects the adverse health effects of non-essential metal ions, notwithstanding the fact that essential metal ions also become toxic at higher concentrations.

A first attempt at integration occurred when a critical amount of information about the molecular structures of metalloproteins became available. The field was then called bioinorganic chemistry with permutations of the name expressing slightly different emphasis: inorganic biochemistry; inorganic biological chemistry; inorganic chemical biology. The main focus of this field is the remarkable chemistry of coordination environments of metals in biomolecules, the discovery of which turned out to be very exciting because in many cases it set precedence for structures and functions hitherto unknown in chemistry. Bioinorganic chemistry includes investigations that may not have biological significance. By and large, it

espouses an emphasis on molecules, and thus, is a reductionist approach. Clearly, after decades of research, in parallel with contemporaneous developments in other fields such as genomics and proteomics, a holistic approach became an attractive way of thinking. Metallomics is such an approach as it addresses the entirety of metal ions in a system rather than individual biomolecules. For example, the knowledge about genomes and proteomes allowed estimating the entirety of metalloproteins in a system, i.e. the metalloproteome.

As an approach to biological systems, metallomics draws attention to the overall significance of metal ions in biology. This development is welcome as the teaching of biochemistry in most textbooks fails to address many of the elements on which life is based and the specific metabolic pathways that handle and control the distribution of the elements in organisms. While this omission was perhaps appropriate when the knowledge about additional bioelements was in its infancy, the current knowledge no longer justifies omission. Historically, the field of biochemistry developed from organic to physiological to biological chemistry. The perceived foundation of biochemistry on organic chemistry was meant to express the fact that life is based on carbon chemistry together with hydrogen, oxygen, and nitrogen chemistry, and to some extent sulphur and phosphorus chemistry. However, associating biochemistry with organic chemistry and non-metals only and thus identifying "organic" with the living world and "inorganic" with the inanimate world is not a useful concept in biochemistry as many more chemical elements than the ones traditionally considered in organic chemistry interact with life or are necessary for life and its evolution. A look at the history of the biometals sciences explains the rather slow evolution of the field and further supports this conclusion.

Investigations of the elemental composition of biological matter date back to at least the beginning of the 19th century. In the case of iron, they can be traced back even further, to the identification of iron in blood in the 18th century (Menghini 1747). The essentiality of zinc

for fungal growth was shown by Jules Raulin, a student of Louis Pasteur, in 1869 (Raulin 1869) in the process of developing industrial fermentation and understanding the nutrient requirements of yeast. However, even as late as in the 1920s it was a matter of conjecture whether the presence of zinc in human tissues is the result of environmental exposure or reflects a functional requirement. Convincing arguments were made for a biological role of zinc in humans (Drinker and Collier 1926). However, its presence in an enzyme, carbonic anhydrase, was not demonstrated until the late 1930s (Keilin and Mann 1939), and it took until the 1960s, 100 years after discovering its essential role for fungal growth, to demonstrate that it is an essential element for humans (Prasad et al. 1961). Since then the field of human zinc biology has advanced to the point where zinc must be considered as important or even more important as iron. At least two reasons can be given why metallobiochemistry was considered a specialty and lagged behind other developments that shaped mainstream biochemistry. The analytical instrumental methods to measure metal ions with sufficiently low detection limits and the ability to address speciation have been developed only rather recently (Maret et al. 2015). Also, knowledge about the complexity of pathways that control cellular metal ion concentrations and re-distribution emerged quite late. Only in the last two decades a critical mass of knowledge allowed appreciating the overall significance of biometals for biochemistry, thus starting to overcome the initially rather skewed assessment of the chemical basis of life.

1.3 Biological Periodic Systems of the Elements

Considering our detailed knowledge today about some aspects of biochemistry, e.g. having sequenced the entire human genome and many other genomes, it is remarkable that the final count on how many chemical elements are essential for life is open, even for humans. For example, bromine was added as an essential element only in 2014 and there are lingering

questions as to whether or not chromium is an essential nutrient (McCall et al. 2014; Mertz 1993; Vincent 2014). It is not widely known that elements such as molybdenum are essential for humans. Only four enzymes use it in humans and they all require the biosynthesis of the pterin cofactor that binds molybdenum to form molybdopterin. Rare diseases leading to molybdenum cofactor deficiency are fatal. Which chemical elements are essential for life is not identical to the question which ones are essential for humans. Some specialized organisms use "unexpected" biometals such as tungsten, cadmium, or even lanthanides (Ljungdahl and Andreesen 1975; Lane and Morel 2000; Pol et al. 2014). The use of nickel and vanadium seems to be restricted to only a few enzymes in certain organisms, not including humans. Biological periodic systems of the chemical elements often do not acknowledge this variation in the usage of the elements in different organisms. For humans, at least 20 chemical elements are essential (Maret 2016b) (Figure 1).

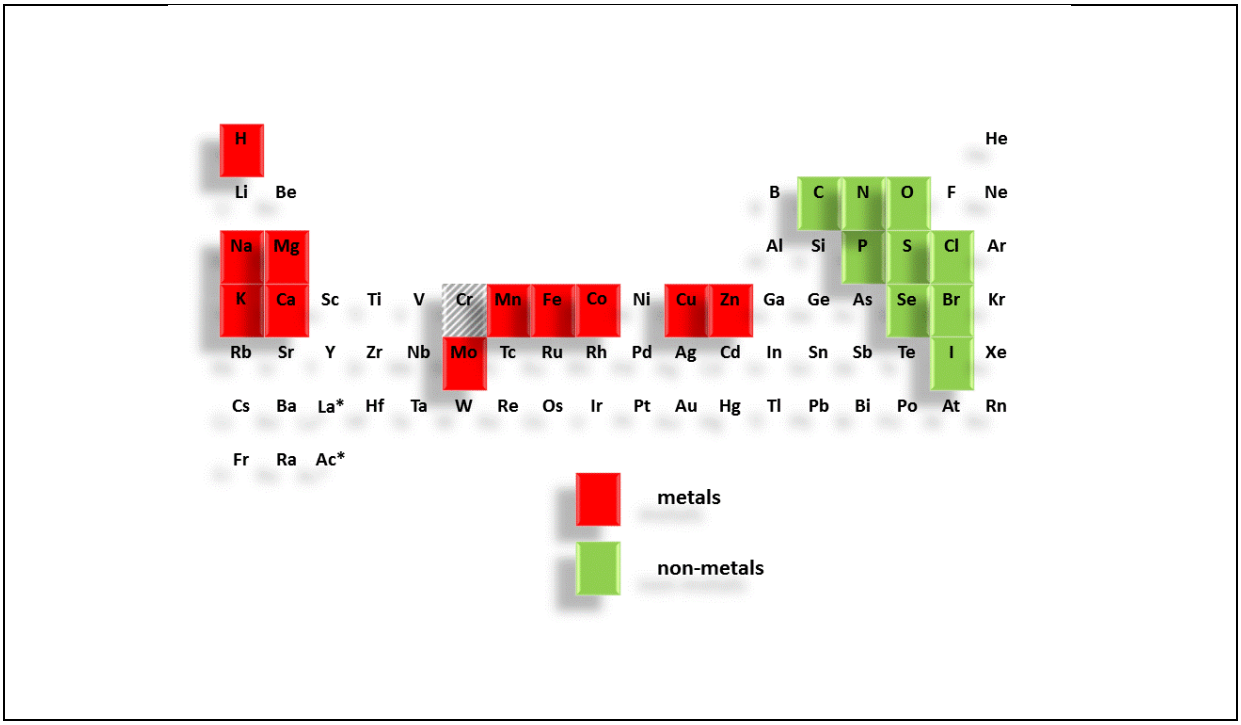

Fig. 1.1 Biological periodic system of the chemical elements that are essential for humans. Chromium is indicated on a background with grey stripes as its status as an essential micronutrient in the form of Cr(III) has been challenged recently.

The definition what "essential" means in this context has undergone some changes. It is important to distinguish whether an element is essential for survival or only important for optimizing a particular function. In some articles, fluorine, which supports tooth health as fluoride, is given as an essential element. However, protecting teeth is not an essential function for life. Fluoride is toxic at rather low concentrations. Some elements, such as the metals nickel and vanadium and the non-metals silicon and boron, are bioactive in certain concentration ranges with some beneficial, but not necessarily essential effects in humans. While molecular mechanisms for their actions are poorly characterized, it is discussed whether intake recommendations for additional not necessarily essential elements should be made (Nielsen 2014a,b,c). It may be relevant that we live with a few hundred different types of commensal bacteria on our mucosal surfaces and skin. In fact, the number of our bacterial cells is higher than the number of our own cells. These bacteria have a different spectrum of essential chemical elements. Thus one reason why some elements are beneficial to us likely relates to the fact that they support the health of our microbiota.

The quantity and relative significance of chemical elements is another area that must be put into proper perspective. Some apparently non-essential elements are present at much higher concentrations than others that are essential (Table). It is not a foregone conclusion that the presence of these elements is without consequence. The same can be said about other non-essential elements that are present at lower concentrations. Employing highly sensitive ICP-MS, the presence of almost any element in a biological sample can be demonstrated: 74 out of the 78 natural elements investigated were measured in salmon egg cells (Haraguchi et al. 2008). Even radioactive elements such as thorium and uranium can be measured in these egg cells and in the human body at relatively high concentrations when compared to

lanthanides for example. In the Table, both essential and non-essential metals are listed according to their abundance in humans.

Table 1.1 Metal composition of the human body (70 kg human) and metal concentrations in the liver and in whole blood. Essential elements are in bold.

	Total amount	Concentration[b,c]	Concentration[b,c]
		Liver (µg/kg)/(ppm)	Whole Blood (µg/L)
Kilogram range			
Calcium	1 kg	--/5100	--/60.5 mg/L
Gram range			
Potassium	140	--/207,000	--/1.62 g/L
Sodium	110/100	--/--	--/1.97 g/L
Magnesium	19	--/13,000	--/37.8 mg/L
Iron	4.2	150-250 mg/kg/16,769	425-500/447 mg/L
Zinc	2.3	40-60 mg/kg/5,543	6-7/7 mg/L
Milligram range[d]			
Rubidium	320/680	400-6000/--	2-4/2.5 mg/L
Strontium	320	--/4	--/31
Lead	120	300-600/122	50-150/214
Copper	72	5000-7000/882	0.8-1.1(m); 1-.4(f)/1.01 mg/L
Aluminium	61/60	0.3-2/65-500	2-8/390
Cadmium	50	500-2000/203	0.3-1.2(ns); 1-4(s)/5.2
Cerium	--/40	--/--	--/--

Barium	22	--/0.2-10	0.5-2.5/<100
Tin	<17/20	100-1000/5-23	<1/<300
Titanium	--/20	--/--	--/<100
Manganese	12	1000-2000/138	8-12/1.6-7.5
Nickel	10/15	10-50/--	1-5?/4.8-106
Gold	10/0.2	--/--	--/0.04-0.42
Molybdenum	9.3/5	400-800/86	1-3/0.95-75
Chromium	1.8/14	5-50/0.7-12.7	<5?/6.5-107
Lithium	--/7	--/--	0.4-1/<40
Cesium	1.5/6	5-20/--	1.5-4.5/<5
Mercury	--/6	30-150/--	2-20/5-20
Germanium	--/5	--/--	--/440-5000
Cobalt	1.5/3	30-150/2-13	5-10/0.3-9.9
Antimony	2	--/--	--/<5
Silver	2	--/0.1-1.7	--/3.4-120
Microgram range			
Uranium	900	--/--	--/<1
Beryllium	360/36	--/--	--/<3.8
Vanadium	--/110	5-20/<1	0.1-0.5/13.6

Footnotes:

ns: non-smokers; s: smokers; m: male; f: female; a human liver weighs 1.2-1.5 kg

a) sources: web2.airmail.net/uthman/elements_of_body, accessed 10/5/2017, which is based on data in Emsley 1998, and for the second values after the slash www.random-science-tools.com/chemical_comp_of_body, accessed 10/5/2017, which is based on data in the CRC Handbook of Chemistry and Physics 1998

b) source: WHO 1996; given as µg/kg = ppb for liver; aside from the different units comparison with the values after the slash cannot readily be made as the former values are not specified with regard to the procedure, i.e. ashed, dried, or fresh liver
c) source: Iyengar et al. 1978 for mean values after the slash and given as mg/kg = µg/g = ppm for ashed liver
d) Additional metals have been measured in the milligram and microgram range; depending on values given from the second source after the slash, the order of abundance may change after titanium; since measurements are from ashed corpses, elements such as mercury and gold used in tooth fillings may contribute to the variation. In addition, there seem to be occasional copying errors and/or issues with the correct units/detection limits, particularly in the case of some of the lower abundance metals.

A number of conclusions can be drawn from this compilation and knowledge about metal metabolism.

1. The quantities of essential metal ions cover approximately six orders of magnitude, from kg (Ca) to g (Na, K, Mg, Fe, Zn) to mg (Cu, Mn, Mo). Thus:

Ca > K,Na > Mg > Fe,Zn > Cu > Mn,Mo > Co.

The amounts of iron (Fe) and zinc (Zn) are in the gram range typical for *minerals* and thus are not really traces. Referring to them as trace metals is not entirely appropriate. In extension of the above terminology, for the studies of *biominerals*, the term *mineralomics* has been proposed (Yasuda et al. 2006). The amounts of the next two essential metals are two orders (copper, Cu) and three orders (manganese, Mn) of magnitude lower, and followed by molybdenum (Mo)/chromium (Cr) and cobalt (Co), which are four orders of magnitude lower. These metals are present as traces. There is no clear definition of the boundaries that separate minerals from trace metals and trace metals from ultratrace metals. The amount of manganese is about the same as that of nickel, which is thought to be non-essential for humans. Remarkably, the amount of vanadium is even lower than that of uranium. The human biochemistry of Mo and Co is linked to cofactor chemistries - corrins and pterins, respectively. But higher amounts of Mo and Co, presumably exceeding the binding

capacities of the cofactors, have a variety of adverse health effects as documented for cobalt (Leyssens et al. 2017) and for molybdenum in the form of molybdosis in ruminants. Mo and Co have very limited usage in humans and are acquired for specific functions as cofactors in as far as we know only in a few human enzymes, two for cobalt in the form of vitamin B_{12} and four for molybdenum in molybdopterin. Manganese is a cofactor in a number of enzymes, but it is unknown how many human proteins require it. Even as late as in 1996, Mn was listed only as "probably essential" for humans (WHO 1996), the reason being that a critical experiment, namely demonstrating essentiality by depleting Mn sufficiently to elicit adverse health effects, has been performed in animals but not in humans. Nevertheless, low Mn status is associated with metabolic syndrome, diabetes, and poor birth outcome in humans and is becoming increasingly an issue due to changes in diets as the primary dietary source of Mn is from plants (Freeland-Graves et al. 2016). On the other hand, Mn toxicity is a concern from natural or contaminated water with high Mn content and from other environmental sources. There seems to be a correlation between the abundance of an essential element and the number of functions it is involved in, giving the field of iron and zinc biology some dominance while making the biology of other, less abundant metal ions more of a specialty, at least for humans.

2. The quantities of non-essential metal ions in biological tissues span a similarly wide range. None are in the range of grams but several are in the milligram range, and importantly, several are at concentrations that are higher than those of some essential metal ions. Noteworthy are the relatively high amounts of rubidium (Rb) and strontium (Sr), where we have very little information about functions, but also those of several others such as alumin(i)um (Al) and titanium (Ti). Thus, with reference to essential elements given in italics:

Rb,Sr > Pb > *Cu*,Al,Cd > Ba,Sn,Ti > *Mn*,Ni > *Co*,U

"Non-essential" should not be interpreted as meaning "non-functional". What is needed is a better understanding of the functional responses of the presence of non-essential elements in organisms, when, where and how they interfere with the functions of essential elements, and which range of their concentrations is acceptable without compromising either our short-term or long-term health. Non-essential metals have their own metalloproteomes, which may partially overlap with the metalloproteomes of essential metals because some non-essential metal ions can bind at sites otherwise occupied by essential metal ions.

3. The uneven distribution of metal ions in biological tissues is a major incentive for research to determine functions, making imaging an attractive approach in metallomics. The uneven distribution is both at the subcellular and organismal levels as, for example, shown in imaging metals in the water flea or the zebrafish (De Samber et al. 2013; Bourassa et al. 2014). Specific tissues need particular metal ions. Examples include calcium in bone and iron in blood, where 90% of all iron is in the hemoglobin of erythrocytes. In contrast to iron, only 1% of zinc is in the blood, making it mostly a cellular ion. Subcellularly, a large part of iron metabolism is linked to mitochondria. The highest concentration of calcium is in the cytosol whereas the highest concentration of zinc is in the nucleus.

4. The distribution of elements between blood and organs shows evidence of active processes in taking up certain metal ions and excluding others. Bioaccumulation factors measured for salmon eggs versus seawater vary over six orders of magnitude (Haraguchi et al. 2008). The essential metal ions (Fe, Zn, Cu, Mn, Co) all have accumulation factors in excess of 10,000 - up to half a million. Some elements that are present at high concentrations in sea water, such as sodium and chloride have negative accumulation factor, i.e. they are excluded in marine (salt water) organisms. Remarkably, the toxic mercury ion also has a bioaccumulation factor of 10,000. Thus, *if present*, it accumulates in biological matter due to the presence of ligands with sulphur donors that have high affinity for mercury ions. There are gradients of metal

ions across the plasma membrane: for potassium the gradient is inward while for sodium the gradient is outward. It is not known how specific these processes of distribution are and whether the presence of non-essential metal ions in cells simply reflects a lack of absolute discrimination, i.e. non-essential metal ions piggy-backing on transport proteins for essential metal ions. Matters of great importance in evaluating the significance of the presence of non-essential metal ions are whether promiscuity was encouraged evolutionarily so that when one metal ion becomes limiting another one can substitute for it and maintain function, whether such metal swapping in proteins is even accompanied with intended functional changes that signal the organism limited availability of the correct metal, or metal substitution merely compromises function.

5. Control of systemic and cellular homeostasis maintains concentrations of essential metal ions in a rather narrow range. Such control minimizes overlap between the functions of different metal ions, is extremely tight for iron and zinc, and is necessary as the specificity of biological coordination environments is not high enough to select only the required metal ion. Thus, *in vitro*, zinc can bind in iron-binding sites of proteins and iron can bind in zinc-binding sites of proteins. The control of availability of metal ions in biological systems provides additional selectivity and avoids that more competitive metal ions bind in sites that need to be populated by less competitive metal ions. For some metal ions, e.g. copper, specific metallochaperones transfer the metal ions to the sites where they are required. The mechanisms of control are quite elaborate and metal-specific, and are part of the many pathways that incorporate chemical elements into biomolecules (Foster et al. 2014). In addition to the metalloproteins that use metals ions for catalytic or structural functions, many metalloproteins are involved in handling and controlling metal ions. The large number of these proteins is further evidence for the importance of pathways for acquisition and

distribution of chemical elements and thus the significant role of metallobiochemistry in general biochemistry (Maret and Wedd 2014).

Other elements that do not underlie such strict homeostatic control as iron and zinc cover a wider range of concentrations in tissues. It appears that essential metal ions are controlled in a narrow range whereas non-essential metal ions, due to a lack of homeostatic control, can vary over a much wider range.

1.4 Metallomics and Human Health

One aim of metallomics is to establish the normal distribution and concentrations of metal ions in tissues and cells. Such reference values then can serve to understand changes that cause disease or are a result of disease. Control of optimal concentrations of metal ions in the human body has huge implications for human health and requires further scrutiny to establish additional guidelines to protect human populations and to prevent and treat disease. Disease can result from a deficiency or an overload of an essential element or the presence of a non-essential and toxic element. Not only the nutritional availability of metal ions determines risk for disease but also the interactions among metal ions, the presence of metal-binding (chelating) agents in the diet and, when patients are medicated, the interactions of metal ions with therapeutic drugs. In addition, genetic variations in the proteins that handle the metal ions affect metal metabolism and utilization. For example, genetic and non-genetic factors can cause iron overload. For zinc, there is no known condition of cellular overload aside from cellular injury. However, there are many genetically determined changes in zinc metabolism, including a condition of high zinc in the blood (hyperzincaemia) due to increased levels of the protein calprotectin (Hogstrand and Maret 2016).

The consequences of deficiencies of essential biominerals, trace and ultratrace biometals on health have been the major focus of investigations but we know comparatively

little what the consequences for human health are, either acutely or chronically, as a result of the presence or accumulation of non-essential metals. It is a very important area of research, as the presence of non-essential metals and the majority of essential metals are never examined clinically on a routine basis. A blood test such as the basic metabolic panel (BMP) contains seven determinations, hence its name CHEM-7 or SMA-7 (sequential multiple analysis). Only sodium and potassium are measured, and sometimes calcium (CHEM-8). While there are seven rather routine tests for iron (ferritin, serum iron, transferrin saturation, total iron-binding capacity, transferrin, transferrin receptor) there is not a single test for zinc or any of the other essential elements, even in extended tests that include liver function, in the so-called comprehensive metabolic panel (CMP), where 14 parameters are determined with SMAC autoanalyzers. Magnesium deficiency is associated with some chronic diseases; there is now evidence for subclinical deficiencies even if the serum magnesium status is normal within the currently accepted reference interval (Costello et al. 2016). We have recommendations for optimal concentrations of the essential elements. However, we often do not have biomarkers that inform us whether the concentrations in tissues and the associated functions are optimal. There should be investigations on the health effects of many more elements aside from the essential and highly toxic ones.

Homeostatic mechanisms control essential but not non-essential elements. The concentrations of non-essential elements are more variable and depend on exposure, interactions, and genetic factors. While UL (upper limit) values have been estimated on the basis of overt signs of toxicity, we do not know all the consequences of the presence of these elements at lower concentrations. Many exposure models are based on a linear relationship between dose and response. However, there are many examples of non-linear responses with hormetic effects. Exposure to some non-essential elements through water, soil, and air has changed drastically in recent years due to new manufacturing practices and using metal

compounds and novel nanoparticles in various high-tech applications. Aside from lanthanides and chemically very reactive tellurium compounds, it includes elements such as hafnium, tantalum, and indium, for which biological investigations of exposure are extremely limited or even non-existent (Ridgway and Webb 2015). Also, metal ions or metal compounds are used as metallodrugs for therapeutic or diagnostic purposes, introducing metals that are usually virtually absent, e.g. Pt-based anticancer drugs, or present at low concentrations, e.g. Li salts for treating manic depression. Gadolinium deposits from gadolinium-based contrast agents used in magnetic resonance imaging (MRI) have been detected in the human brain and bone, resulting in a "gadolinium deposition disease"/"gadolinium toxicity disorder", which is presently being further evaluated (Ramalho et al. 2016). On the other hand, we are exposed to chelating agents such as some nutritional supplements or food additives and pharmaceuticals that bind metal ions and interfere with metal metabolism. Also, changing diets in many countries due to new ways of food production and processing and changes in food preferences and availability have additional effects on the nutritional status of essential and non-essential elements.

Some mechanisms of detoxification for non-essential elements have been discussed. There is a wide range of toxicities with different modes of action. Lead (Pb), for example, is a very potent inhibitor for δ-aminolevulinic acid dehydratase interfering with heme biosynthesis. In contrast, the actions of cadmium (Cd) seem to be pleiotropic as a large number of proteins interact with it (Maret and Moulis 2013). The amounts of cadmium and lead in the human body are remarkably high, in the order of those of essential copper and presumably non-essential nickel. For lead, it is now becoming accepted that there is no threshold value for its toxicity and that no level of exposure can be considered safe (Maret 2017). For cadmium, such a relationship between exposure and health effects has not been established.

The description of functional effects would be incomplete without pointing out the significance of interactions. Thus, in many instances the presence or absence of one biometal influences the presence or absence of another biometal. For example, under iron deficiency, more cadmium is taken up, making deficiencies of essential metal ions a risk factor for toxic metal ions. The availability of a metal ion may be influenced by the nutritional status of a non-metal, e.g. selenium. Requirements for essential metal ions and vulnerability to the toxic effects of metals ions depend on life cycle stages. Toxic metals such as lead and cadmium accumulate with age. There is evidence of dyshomeostasis of essential metal ions in older people. Apparently, homeostatic control works less efficiently and uncomplexed (free) metal ions accumulate and interfere with protein functions. One effect is pathological protein aggregation triggered by metal binding to proteins. Neurodegenerative diseases such as Alzheimer's Disease is one example where such a role of metal ions has been demonstrated.

Each element requires biochemical investigations to determine the range of concentrations in healthy and diseased human tissue. There should be increased monitoring, which is relatively inexpensive for metals compared to organic molecules, as metal ions are relatively easy to identify and quantify without further separation techniques.

1.5 Bioinformatics: Predicting Metalloproteomes

Most of our present knowledge of metallobiochemistry relates to the interaction of metal ions with proteins, with the notable exception of magnesium biochemistry pertaining to the interaction with RNA. Accordingly, a major emphasis in metallomics is on metalloproteomes and metalloproteomics (Maret 2010). The combination of experimental and computational approaches has been very successful and together with advances in analytical techniques has laid the foundations for the field of metallomics as a systems biology approach. Once a sufficient number of sequences and 3D structures of

metalloproteins was determined, analyses of the metal-binding sites revealed some general features. It was recognized, for example, that the binding of calcium in EF hands and zinc in zinc fingers is similar in different proteins, as reflected in the spacing and types of ligand donors in the primary structure (sequence) (Kretsinger and Barry 1975; Miller et al. 1985). Recurring patterns or "signatures" of amino acids in sequences were recognized, which then could be employed as templates for identifying unknown metal binding sites in other proteins. One factor is the type of amino acid that provides the donor ligand to the metal. Only a few amino acids have such donors in their site chain: oxygen from Glu, Asp, nitrogen from His, and sulphur from Cys, and in some instances oxygen from Tyr (Fe) and sulphur from Met (Cu). Rarely others, such as oxygen from serine, serve as ligand donors. Another critical observation was that short spacers of 1-4 amino acids - or no spacer at all - occur between amino acids that provide the donors and form the metal-binding sites (Figure 2). In metalloenzymes, usually a long spacer follows the short spacer to reach the amino acid that provides the third ligand donor (Vallee and Auld 1989). In structural metal sites, the spacers are usually shorter. The number of amino acids in the spacers is often conserved, as are, to some extent, the types of amino acids. There are exceptions to these rules, though. When inspecting the tertiary structures of proteins, it was noticed that domains for metal binding are conserved in a large number of metalloproteins. This modular nature of metal binding sites in proteins is also employed for prediction, either in queries for unknown metalloproteins when using protein or nucleic acid sequences or in queries of entire genomes, which became available in increasing numbers (Andreini et al. 2011). Several web servers are available for performing predictions.

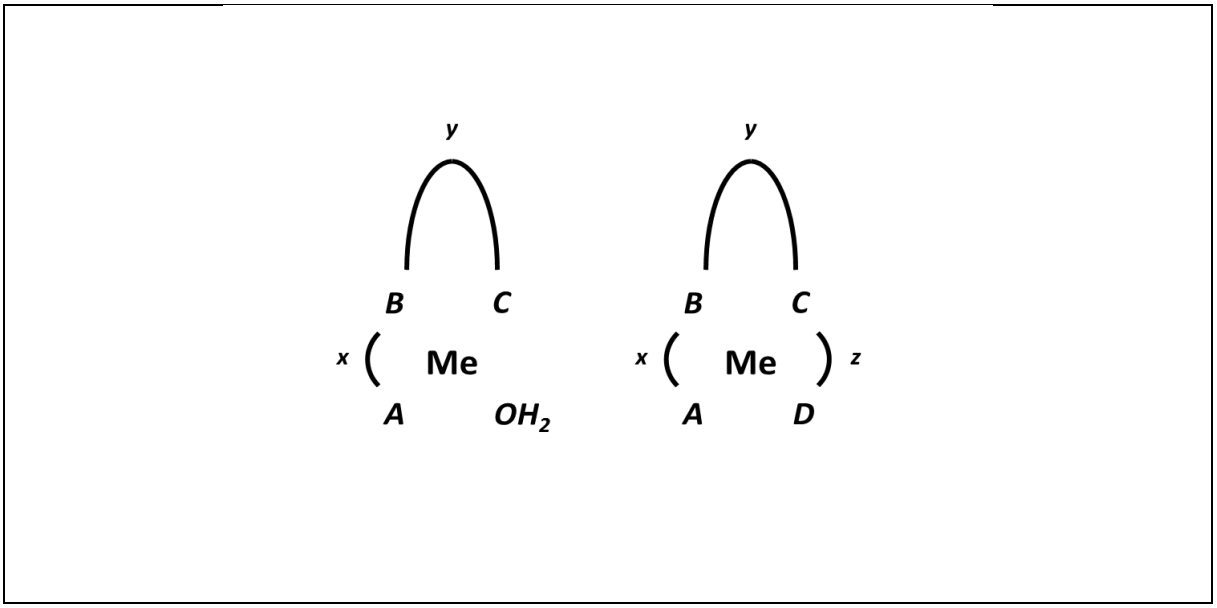

Fig. 1.2 Metal signatures in proteins. The amino acids that provide the ligand donors (A,B,C,D) to the metal (Me) are separated by characteristic spacers (x,y,z). Left: catalytic metal site in enzymes, where the fourth ligand donor is a water molecule in the substrate binding site. Right: structural metal site in proteins. The presentation should be understood as an abstract cartoon only to illustrate the concept of how spacers and ligand donors are employed to predict metal sites in proteins. In reality, there is considerable variation in the number of ligand donors (coordination number) and the lengths of spacers.

While this novel way of searching for metalloproteins employing bioinformatics and mining databases turned out to be remarkably successful, the classical and labour-intensive approach of isolating and characterizing metalloproteins is still needed for experimental verification of the predictions. The bioinformatics approach made it possible to predict the total number of metalloproteins for some essential metals, the metalloproteomes, in different organisms. It allowed estimating the number of iron, copper, and zinc proteins, but not manganese proteins due to the lack of suitable consensus signatures. The bioinformatics approach made an enormous contribution to the field because it increased the number of known metalloproteins significantly, at least by an order of magnitude, and thus, for the first time, showed the full impact of metals in biochemistry: 1% of all human proteins contain non-heme iron plus about 70 proteins Fe-S clusters, 9% zinc, and 1% copper (Andreini et al.

2009; Andreini et al. 2016). If one adds calcium, magnesium, and heme proteins, an estimated 30-40% of all proteins require a metal cofactor. Thus, in humans, there are more than 3000 zinc proteins and 54 copper proteins (Blockhuys et al. 2017). The mining of genomes using metal signatures introduced an omics approach to metallobiochemistry for investigating the entirety of metalloproteins. A variant of this approach is the search for selenoproteins in genomes. Selenium, a chemical homologue of sulphur, can be present in the inorganic ionic form or covalently bound in many chemical species and with various valence states. It is incorporated into selenoproteins as selenocysteine by recoding the stop codon and using a stem-loop structure in the mRNA and a couple of ancillary proteins. Twenty human selenoproteins are known (Lu and Holmgren 2009). Selenium from selenocysteine can also be a ligand donor to metal ions. Selenomethionine, on the other hand, is incorporated into proteins randomly.

The specificity of signatures is linked to the factors that determine coordination environments of biometals in proteins, which is a mainstay area of bioinorganic chemistry/structural biology. For calcium, which is an important cellular signalling ion (2nd messenger), remarkably specific sites have evolved. To perform the functions of calcium, biology had to make coordination environments that bind calcium with higher affinity than magnesium, opposite to general chemical principles according to which the affinity of magnesium for the same ligands is higher. Selectivity was achieved by using up to seven donor atoms from ligands in sites and also a unique amino acid, γ-carboxyglutamic acid (Gla), which is synthesized in a vitamin K-dependent reaction.

The bioinformatics approach to metalloproteomes of essential metal ions has some limitations as discussed for the zinc proteome (Maret 2004, 2008): i) it is a prediction and not a chemical analysis; ii) it can employ only known signatures for prediction, clearly leaving out metalloproteins for which signatures are yet unknown or are not recognizable in

sequences because the ligands differ or have different spacer lengths; iii) it *assumes* that the signature binds a specific metal ion. The latter point is critical because the metal ion may not be present at all, as the availability of metal ions is determined by the entire regulatory systems of the organism responsible for distributing and allocating metal ions and not just the binding capacity of a site. The availability of metal ions is variable and hence the metalloproteome is variable. At low availability, some sites will not be occupied while at high availability additional sites will bind metal ions. In some sites, the metal binds only transiently and hence the metal may not be present when the protein is isolated. There is also ambiguity concerning which metal ion is bound. A particular signature can bind one metal in one instance and another one in another instance. This promiscuity is known for manganese and iron superoxide dismutase and other cambialistic proteins. Another example is metallothionein, a family of proteins in humans, binding zinc, copper, cadmium and less abundant metal ions (Krężel and Maret 2017). Other limitations include: iv) prediction is possible for certain structural and catalytic sites but not for inhibitory sites, intersubunit sites, where the ligand donors are on different proteins, or sites where the ligand donors are far apart from each other in the sequence. Signatures are linear, sequential motifs of amino acids. However, there are instances where the ligand donors are arranged non-sequentially or where the donors form ligand bridges. Such binding is difficult or impossible to predict. Lastly, v) predictions are looking only at metal binding to proteins. The metalloproteome is not identical with the metallome. The difference between metals bound to proteins and metals bound to other biomolecules increases when the metal ions become less competitive. Less competitive metal ions are distributed in a wider pool of biomolecules because the equilibrium between protein-bound and free metal ion leaves much more free (non-protein bound) metal ions to bind to other species. Even for a highly competitive metal ion such as Zn^{2+} the pool of non-protein bound cellular zinc is not negligible and has a significant role in

cellular regulation as a signalling ion. Accordingly, it requires regulatory mechanisms in addition to the control of cellular homeostasis (Maret 2013; 2014). Metal buffering involving metal-binding biomolecules and transport of metal ions through the cytoplasmic membrane or subcellular membranes controls free metal ion concentrations. The dynamic biological transport processes contributing to biological metal buffering are referred to as muffling (Colvin et al. 2010).

1.5The Multidimensionality of Metallomics

Metallomics can be subdivided into structural, functional, and quantitative metallomics. Structural metallomics addresses the chemical structures of metal ions and metalloids. Functional metallomics aims at annotating these structures in terms of their biological functions. Quantitative metallomics determines the concentrations of metal ions and metalloids in tissues and cells, including imaging their distribution in two and three dimensions and overlaying such images with physical maps obtained with microscopy and computer tomography techniques. Comparative genomics of metallomes focuses on the utilization of metals and evolutionary dynamics in different forms of life (Gladyshev and Zhang 2013).

Metallomes are inherently incomplete and that there are an infinite number of metallomes depending on the biological context. Biological systems are dynamic, the essence of being alive, with active metabolism and changes occurring in biological space and time. Every individual is slightly different. We observe this in genetics with the *variome*, a set of genetic variations in populations of one species, some of which affect metal metabolism and distribution, and we see this in non-genetic factors, the *phenome*, a sum of all phenotypic traits. Individual variation is of course present in metallomes due to

environmental factors such as nutrition, exposure, and disease. This is the area of *metametallomics,* analogous to the field of metagenomics, the study of genetic material dependent on all environmental influences.

1.6 Conclusion

Metallomics emphasizes the importance of metals in biology through the integration of research fields and methodologies. It is an emerging multi-disciplinary field of research, and when applied properly has far-reaching implications and future potential. The interpretation of metallomics data requires training in biology and medicine, which is not part of the traditional education of a chemist. Thus additional curricular activities and training opportunities are necessary for the field to prosper. It took considerable time and effort to arrive at this point and will require such for further development.

References

Andreini C, Bertini I, Rosato A (2006) Metalloproteomes: A bioinformatic approach. Acc Chem Res 42:1471–9

Andreini C, Banci L, Rosato A (2016) Exploiting bacterial operons to illuminate human iron-sulfur proteins. J Proteome Res 15:1308-22

Andreini C, Bertini I, Cavallaro G et al (2011) A simple protocol for the comparative analysis of the structure and occurrence of biochemical pathways across superkingdoms. J Chem Inf Model 51:730-8

Baker M (2013) The 'omes puzzle. Nature 494:416-19

Blockhuys S, Celauro E, Hildesjö C et al (2017) Defining the human copper proteome and analysis of its expression variation in cancers. Metallomics 9:112-23

Bourassa D, Gleber S-C, Vogt S et al (2014) 3D imaging of transition metals in the zebrafish embryo by X-ray fluorescence microtomography. Metallomics 6:1648-55

Colvin RA, Holmes WR, Fontaine CP et al (2010) Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis. Metallomics 2:306-17

Costello RB, Elin RJ, Rosanoff A et al (2016) Perspective: The case for an evidence-based reference interval for serum magnesium: The time has come. Adv Nutr 7:977-93

CRC Handbook of Chemistry and Physics (1998) 79th edition, Lide DR, ed., CRC Press:Boca Raton, FL

Cvetkovic A, Menon AL, Thorgersen MP et al (2010) Microbial metalloproteomes are largely uncharacterized. Nature 466:779–84

De Samber B, Evens R, De Schamphelaere G et al (2008) A combination of synchrotron and laboratory X-ray techniques for studying tissue-specific trace level metal distributions in *Daphnia magna*. JAAS 23:829-39

Drinker KR, Collier ES (1926) The significance of zinc in the living organism. J Industr Hygiene 8:257-69

Emsley J (1998) The Elements, 3rd edition, Clarendon Press:Oxford

Foster AW, Osman D, Robinson NJ (2014) Metal preferences and metallation. J Biol Chem 289:28095-103

Freeland-Graves JH, Mousa TY, Kim S (2016) International variability in diet and requirements of manganese: causes and consequences. J Trace Elem Med Biol 38:24-32

Gladyshev VN and Zhang Y (2013) Comparative Genomics Analysis of the Metallomes, in "Metallomics and the cell", L. Banci, Guest Ed.; Vol. 12 of 'Metal Ions in Life Sciences', A. Sigel, H. Sigel, R. K. O. Sigel, Eds.; Springer Science + Business Media B.V., Dordrecht, The Netherlands, p. 529-80

Haraguchi H (2004) Metallomics as integrated biometal science. JAAS 19:5-14

Haraguchi H, Ishii A, Hasegawa T et al (2008) Metallomics study on all-elements analysis of salmon egg cells and fractionation analysis of metal in cell cytoplasm. Pure Appl Chem 80:2595-2608

Haraguchi H (2017) Metallomics: The history in the last decade and the future outlook. Metallomics 9:1001-13

Hogstrand C, Maret W (2016) Genetics of human zinc deficiencies. In: eLS. John Wiley & Sons, Ltd:Chichester, DOI: 10.1002/9780470015901.a0026346

Iyengar GV, Kollmer WE, Bowen HJM (1978) The elemental composition of human tissues and body fluids, Verlag Chemie:Weinheim

Keilin D, Mann T (1939) Carbonic Anydrase. Nature 144:442-3

Kretsinger RH, Barry CD (1975) The predicted structure of the calcium-binding component of troponin. Biochim Biophys Acta 405:40-52

Krężel A, Maret W (2017) The functions of metamorphic metallothioneins in zinc and copper metabolism. Int J Mol Sci 18:1237

Lahner B, Gong J, Mahmoudian M et al (2003) Genomic scale profiling of nutrient and trace elements in *Arabidopsis thaliana*. Nat Biotechnol 21:1215-21

Lane TW, Morel FMM (2000) A biological function for cadmium in marine diatoms. Proc Natl Acad Sci USA 97:4627–31

Leyssens L, Vinck B, Van Der Straeten C et al (2017) Cobalt toxicity in humans-A review of the potential sources and systemic health effects. Toxicology 387:43-56

Li Y-F, Chen C, Qu Y et al (2008) Metallomics, elementomics, and analytical techniques. Pure Appl Chem 80:2577-94

Ljungdahl LG, Andreesen JR (1975) Tungsten, a component of active formate dehydrogenase from *Clostridium thermoaceticum*. FEBS Lett 54:279-82

Lobinski R, Becker JS, Haraguchi H et al (2010) Metallomics: Guidelines for terminology and critical evaluation of analytical chemistry approaches (IUPAC technical report). Pure Appl Chem 82:493-504

Lu J, Holmgren A (2009) Selenoproteins. J Biol Chem 284:723-7

Maret W (2004) Exploring the zinc proteome. J Anal At Spectrom 19:15-9

Maret W (2008) Zinc proteomics and the annotation of the human zinc proteome. Pure Appl Chem 80:2679-87

Maret W and Li Y (2009) Coordination dynamics of zinc in proteins. Chem Rev 109:4682-4707

Maret W (2010) Metalloproteomics, metalloproteomes, and the annotation of metalloproteins. Metallomics 2:117-25

Maret W (2013) Zinc and the Zinc Proteome, in "Metallomics and the Cell", L. Banci, Guest Ed.; Vol. 12 of 'Metal Ions in Life Sciences', A. Sigel, H. Sigel, R. K. O. Sigel, Eds.; Springer Science + Business Media B.V., Dordrecht, The Netherlands, p. 479-501

Maret W and Moulis J-M (2013) The Bioinorganic Chemistry of Cadmium in the Context of its Toxicity, in "Cadmium: From Toxicity to Essentiality", Vol. 11 of 'Metal Ions in Life Sciences', A. Sigel, H. Sigel, R. K. O. Sigel, Eds.; Springer Science + Business Media B.V., Dordrecht, The Netherlands, p. 1-29

Maret W (2014) Molecular aspects of zinc signals, in: Zinc signals in cellular functions and disorders, Fukada T, Kambe T, eds. Springer:Japan, p. 7-26

Maret W, Wedd AG, eds. (2014) Binding, Transport and Storage of Metal Ions in Biological Cells. Royal Society of Chemistry:Cambridge

Maret W, Caruso JA, Contag CH et al (2015) in: Trace Metals and Infectious Diseases, Nriagu JO, Skaar EP, eds. MIT Press:Cambridge, p. 341-401

Maret W (2016a) Metallomics, a Primer to Integrated Biometal Sciences, Imperial College Press:London

Maret W (2016b) The metals in the biological periodic system of the elements: concepts and conjectures. Int J Mol Sci 17:66

Maret W (2017) The bioinorganic chemistry of lead in the context of its toxicity, in: "Lead – Its effects on environment and health", Vol. 17 of 'Metal Ions in Life Sciences', A. Sigel, H. Sigel, R. K. O. Sigel, Eds.; W. de Gruyter, Berlin:Germany, p. 1-20

McCall AS, Cummings CF, Bhave G et al (2014) Bromine is an essential trace element for assembly of collagen IV scaffolds in tissue development and architecture. Cell 157:1380–92

Menghini V (1747) De Ferreorum particulerum sede in sanguis. Commentar. Bononiens (Bologna)ii, p. 475

Mertz W (1993) Chromium in human nutrition. J Nutr 123:626–33

Miller J, McLachlan AD, Klug A (1985) Repetitive zinc-binding domains in the protein transcription factor IIIA from Xenopus oocytes. EMBO J 4:1609-24

Nielsen FH (2014a) Should bioactive trace elements not recognized as essential, but with beneficial health effects, have intake recommendations. J Trace Elem Med Biol 28:406–8

Nielsen FH (2014b) Update on the possible nutritional importance of silicon. J Trace Elem Med Biol 28:379–82

Nielsen FH (2014c) Update on human health effects of boron. J Trace Elem Med Biol 28:383–7

Pol A, Barends TRM, Dietl A et al (2014) Rare earth metals are essential for methanotrophic life in volcanic mudpots. Env Microbiol 16:255–64

Prasad AS, Halsted JA, Nadimi M (1961) Syndrome of iron deficiency anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med 31:532-46

Ramalho J, Ramalho M, Jay M et al (2016) Gadolinium toxicity and treatment. Magn Reson Imaging 34:1394-98

Raulin J (1869) Etudes chimiques sur la végétation. Ann Sci Nat Bot Biol Veg 11:92-299

Ridgway A and Webb R (2015) The materials bonanza. New Sci 225:35-41

Salt DE (2004) Update on ionomics. Plant Physiol 136:2451-56

Szpunar J (2004) Metallomics: a new frontier in analytical chemistry. Anal Bioanal Chem 378:54-6

Vallee BL, Auld DS (1989) Short and long spacer sequences and other structural features of zinc binding sites in zinc enzymes. FEBS Lett 257:138-40

Vincent JB (2014) Is chromium pharmacologically relevant? J Trace Elem Med Biol 28:397–405

WHO (1996) Trace elements in human nutrition and health, World Health Organization Geneva, p. 258-9

Williams RJP (2001) Chemical selection of elements by cells. Coord Chem Rev 216:583-95

Yasuda H, Yonashiro T, Yoshida K et al (2006) Relationship between body mass index and minerals in male Japanese adults. Biomed Res Trace Elem 17:316-21

Zhang P, Georgiou CA, Brusic V (2017) Elemental metabolomics. Briefings in Bioinformatics, DOI: 10.1093/bib/bbw131